

SEXUAL HORMONES IN *ACHLYA*. VI. THE HORMONES OF THE *A-COMPLEX**

BY JOHN R. RAPER

DEPARTMENT OF BOTANY, UNIVERSITY OF CHICAGO

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The mechanism of hormonal control of sexual processes in heterothallic species of *Achlya* comprises a number of successive stages, each exhibiting complete dependence on that immediately preceding it.¹ Four morphological phases, each initiated and controlled by one or more specific hormones, are recognized: (1) the production of antheridial hyphae on the ♂ plant in response to the hormones of the *A-Complex* secreted by the vegetative ♂ and ♀ plants; (2) the production of oogonial initials on the ♀ plant in response to hormone *B* secreted by the antheridial hyphae; (3) the chemotropic attraction of antheridial hyphae to oogonial initials and the differentiation of antheridia in response to hormone *C* secreted by the oogonial initials; and (4) the delimitation of oogonia and the differentiation of the oogonial contents to form oöospheres in response to hormone *D* secreted by differentiated antheridia. The dual roles of hormones *C* and *D* indicate the probability that each is a hormonal complex consisting of two or more specific substances. Thus the interchange between the two sexes throughout the entire sexual reproductive process consists of specific, diffusible substances, with the exception of the physical transfer of ♂ nuclei in fertilization.

It was recognized early in the work that the ultimate understanding of the correlative mechanism must depend upon the stepwise elucidation of the successive stages, with concurrent development of the ability to control exactly those stages in the chain of events prior to that of immediate interest. A large part of the work has, therefore, been concentrated upon the initiation of the entire sexual progression (the production of antheridial hyphae) and the factors which quantitatively affect this response.^{2, 3} Continued work has furnished more exact detail concerning the activities of the hormones previously described and has revealed that two new hormones, one secreted by the vegetative ♀, the other by the vegetative ♂, are involved in the control of antheridial hyphal production. The activities of these two new hormones, particularly in combination with those previously described, necessitate a revision of the description of that portion of the hormonal mechanism pertaining to the production of antheridial hyphae.

The work reported here has been done with new isolates of ♂ and ♀ strains of *Achlya bisexualis* and *A. ambisexualis*.⁴ All major results of the work prior to 1942 have been confirmed with these new isolates and it is thereby known that they are comparable with the isolates originally

employed. The testing methods for hormones affecting the production of antheridial hyphae have been described in detail elsewhere.^{2, 3} The hormone concentrates which have been used include: hormone *A* "standard" solution in acetone containing 5×10^5 U./ml., prepared and standardized in 1948 from partially purified and highly concentrated material from filtrates of *A. bisexualis* ♀ in 1943, and acetone-soluble and water-soluble (acetone-insoluble) fractions of filtrates from ♂ and ♀ strains of both heterothallic species. These latter fractions were prepared from each of the filtrates by the following procedure: diatomaceous earth, "Celite," was added to the filtrate and the water was removed from the slurry by distillation *in vacuo*; the dried material, deposited on the inert filler, was then exhaustively extracted with acetone in a Soxhlet's extraction apparatus to provide the acetone-soluble fraction; and the acetone-insoluble material on the filler was recovered by solution in water.

The initiation of the formation of antheridial hyphae on the ♂ plant occurs within an hour after the introduction of the test plants into water containing the appropriate hormones in the proper concentrations. By the end of three hours the antheridial hyphae are 100–500 μ in length and may be counted easily. Each vegetative hypha is treated as an individual and the average number of antheridial hyphae/3 mm. hyphal tip is taken as an index of the intensity of the reaction. Twenty to 100 or more individuals are counted for each test, the number depending upon the degree of accuracy required for the immediate purpose.

Four different hormones, collectively designated the *A-Complex*, are now known to affect quantitatively the production of antheridial hyphae. Two hormones, *A* and *A*², are secreted by the vegetative ♀ mycelium and each alone is capable of inducing the response. Two other hormones, *A*¹ (previously designated *A'*)² and *A*³, are secreted by the vegetative ♂ mycelium and neither can initiate the reaction. Since hormones *A* and *A*³ are readily soluble in acetone or dioxane while hormones *A*¹ and *A*² are not, quantitative separation of the various hormones is readily accomplished.

The different hormones of the *A-Complex* will be considered in the order of their discovery.

Hormone *A*, secreted by the ♀, induces its characteristic response, the production of antheridial hyphae, with an intensity which is a logarithmic function of its concentration (the curve at the lower left of Fig. 1). This relationship obtains over a concentration range from 0.05 to 5000 U./ml., a factor of 100,000. Antheridial hyphae averaging fewer than 30/hyphal tip are most easily and accurately counted. Since maximal responses of this intensity are induced by approximately 50 U./ml., a greatly restricted range of concentrations, 0.5–50 U./ml., is therefore commonly employed.

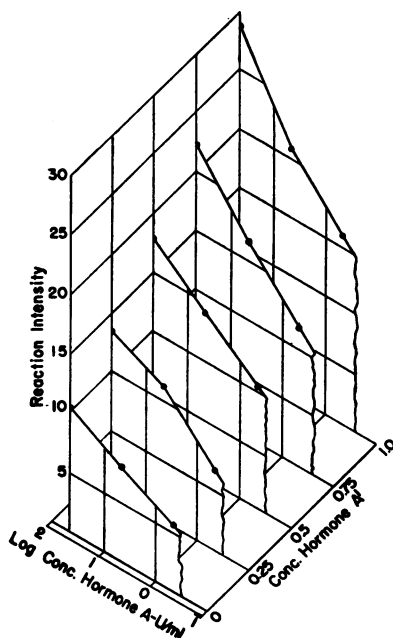


FIGURE 1

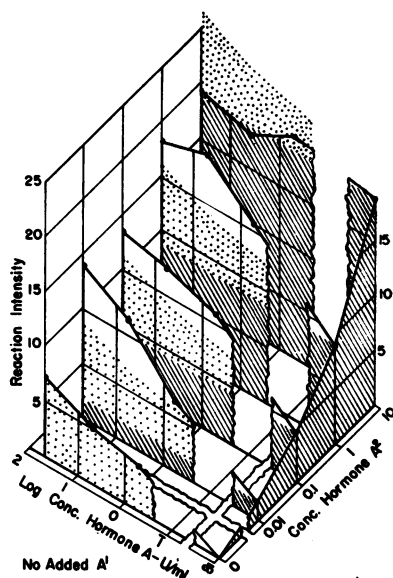


FIGURE 3

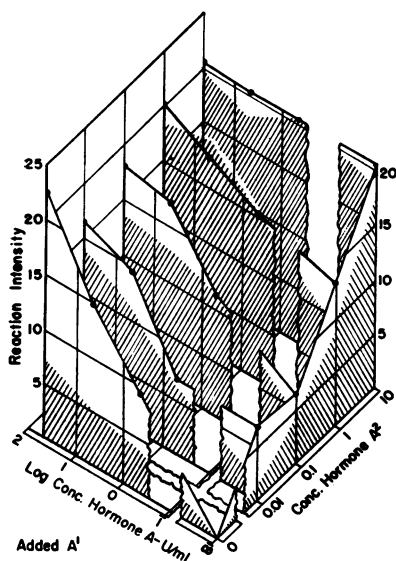


FIGURE 4

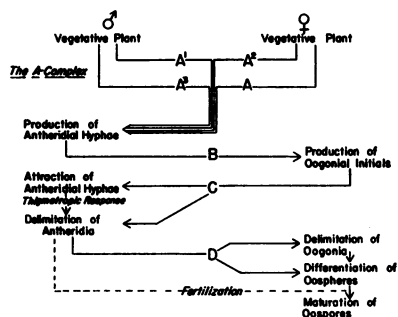


FIGURE 6

See opposite page for description of illustrations.

Hormone A^1 , which is secreted by the vegetative σ^7 and has no initiating activity of its own, markedly enhances the intensity of the reaction to hormone A . The pattern of effect of A^1 on reaction intensity, at three concentrations of hormone A , is shown by the four upper curves of figure 1, representing four different concentrations of A^1 . The responses elicited by hormone A in the absence of added A^1 is shown by the curve at the lower left of figure 1. The data plotted in figure 1 represent the average values for four tests, each point being the average reaction of 80 individual hyphae. From the data given in figure 1 and from the results of other series not included here, it is apparent that for a given concentration of hormone A an optimal concentration of hormone A^1 increases the intensity of the reaction by a factor of about three. For suboptimal concentrations of A^1 , concentration of A remaining constant, the reaction intensity is roughly a linear function of A^1 concentration. The concentration range of A^1 over which this relationship holds, however, is very restricted as compared to that of hormone A ; the greatest concentration of A^1 used here, that of raw filtrate from a mass culture of σ^7 mycelium, approaches an optimal value. Low concentration of either A or A^1 acts to limit the response. An intense production of antheridial hyphae is therefore possible only if both hormones are present in adequate amounts.

No method has yet been devised to determine whether A^1 is indispensable for the production of antheridial hyphae since the only means of testing for the hormone depends upon the reaction of the plant which simultaneously secretes it. Previous work has shown that amounts of A^1

Figure 1. Production of antheridial hyphae on the σ^7 plant in relation to graded concentrations of hormones A (φ) and A^1 (σ^7) when the two hormones are independently varied. In this and in subsequent figures the average number of antheridial hyphae/-3 mm. hyphal tip produced within four hours following the introduction of σ^7 plants into the test solutions is used as the index of reaction intensity and the concentrations of hormone A tested were 50, 5 and 0.5 U./ml.

Figure 3. Response, in the absence of added hormone A^1 , to graded concentrations of hormone A alone (stippled curve at lower left) and hormone A^2 alone (lined curve at lower right) and to independently varied concentrations of both A and A^2 (four enclosed composite curves). The additive values for specific combinations of A and A^2 are indicated by the superposition of the curve for hormone A alone (stippled) on the projection of the control reaction for A^2 alone (lined) across each curve. Thus the unshaded portion of each curve represents the mutually augmentative effect of the two hormones in combination in excess of the sum of their separate activities.

Figure 4. The effect of added hormone A^1 on the responses induced by hormones A and A^2 in the same concentrations shown in figure 3. The response values for the various A plus A^2 combinations are shown by the shaded portions of each curve. Thus the unshaded portion, if any, of each curve represents the augmentative effect of hormone A^1 on the combined activity of hormone A plus A^2 .

Figure 6. Revised diagram of the hormonal mechanism which initiates and coordinates the several phases of the sexual process in heterothallic species of *Achlya*.

significantly affecting the reaction are secreted by the ♂ plant during the 3–4-hour period required for the test.² The quantity of A^1 secreted into the test solution has also been shown to vary rhythmically, each cycle extending over 18–19 hours. Reasonably reproducible reactions may be obtained to a given concentration of hormone A over extended periods, however, if hormone A^1 is added in non-limiting quantity.

Hormone A^2 , the third specific secretion involved in the control of the production of antheridial hyphae, is secreted simultaneously with hormone A by the vegetative ♀ and is chemically separable from hormone A . Hormone A^2 , like A , induces the production of antheridial hyphae in the absence of other added hormones. The pattern of the response intensity

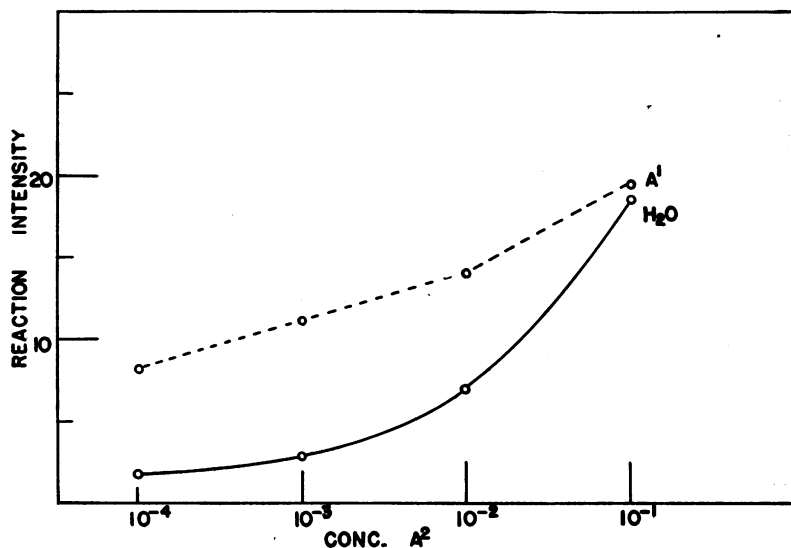


FIGURE 2

Response to graded concentrations of hormone A^2 (♀) in the absence (unbroken curve) and presence (broken curve) of added A^1 .

vs. hormonal concentration is quite distinct from that of hormone A , the reaction intensity being roughly an exponential function⁵ of the concentration of hormone A^2 (Fig. 2 and lower right curve of Fig. 3). The addition of hormone A^1 augments the response elicited by graded concentrations of hormone A^2 (Fig. 2). The augmentative effect of A^1 on A^2 , however, does not resemble that on hormone A . Instead of increasing the response by a constant factor as with hormone A , its effect is most pronounced with low concentrations of A^2 and diminishes with increasing concentration of A^2 , having but little effect when the latter is present at ten times its concentration in raw filtrate.

A still different pattern of augmentation results when hormones A and A^2 are combined. A plot of reaction intensity as a function of concentration of hormones A and A^2 , when the concentrations of the two hormones are independently varied, is given in figure 3. Each of the two hormones is able, in the absence of the other, to induce the response in the intensities shown by the curves at the lower left and lower right of the figure. The results which obtain in other portions of the plot, i.e., when both A and A^2 are simultaneously present, depend upon both the absolute quantities and the relative concentrations of the two hormones. For example, when A^2 is present in a concentration of 0.01, the response intensity increases markedly with increase in the concentration of A , the reaction in each combination of A and A^2 being higher than the sum of the reactions separately induced by the two hormones. The additive values for all combinations of A and A^2 are indicated in figure 3 by the superposition of the control response curve to hormone A alone (stippled) above the projection of the response to hormone A^2 alone (lined) across the face of each curve. The greater-than-additive effectiveness of the A plus A^2 combinations (the unshaded portions of each curve) is thus seen to obtain in all cases except at the highest concentration of A^2 . Actually, the relationship shown in the three central curves of figure 3 probably obtains throughout the concentration ranges of both hormones found in matings of ♂ and ♀ plants. The highest concentration of A^2 used here, $10 \times$, would almost certainly never be encountered in a filtrate of ♀ plants or in the liquid in which plants are mated; the concentrations of hormone A used here, however, fall within the range commonly present under mating conditions.

The response induced by combining hormones A and A^2 is further augmented by the addition of an optimal concentration of hormone A^1 . The pattern of this augmentation is shown in figure 4, in which reaction intensity (vertical coordinates) is plotted against independently varied concentrations of hormone A and A^2 in the presence of hormone A^1 . In this figure the reactions elicited by the specific combinations of A and A^2 in the absence of added A^1 (taken from the data presented in Fig. 3) are shown by the shaded areas on each curve. Thus the augmentative effect of A^1 for the various combinations of A and A^2 is indicated by the unshaded areas on the several curves. The augmentation by hormone A^1 of the response in the various combinations of the hormones A and A^2 is, however, less marked than its effect on either of the ♀-secreted hormones alone and it is greatly reduced in the presence of high concentrations of hormone A^2 . Actually, in certain combinations, the augmentative capacity of hormone A^1 would appear to be definitely decreased by hormone A^2 , even in low concentrations of the latter. Thus the response to 50 U./ml. of hormone A plus A^1 is reduced from an average number of 22.5 antheridial hyphae/vegetative hypha to 16.1, 17.6, 20.1 and 20.4 by the addition of hormone

A^2 in concentrations of 0.01, 0.1, 1.0 and 10, respectively. Each of these points is based on the counts of 80–200 individual reacting hyphae and the decrease in each case due to hormone A^2 is unquestionably significant. This inhibitory effect is present, however, only at the single highest concentration of hormone A , 50 U./ml.⁶

Hormone A^3 , the fourth secretion involved in the quantitative control of the production of antheridial hyphae, has been studied less intensively than the three hormones described above. This is especially true of the complex interactive effects in the various possible combinations with the other hormones. Hormone A^3 is secreted by the vegetative σ^1 plant simultaneously with hormone A^1 , from which it is easily separable because of its ready solubility in acetone and dioxane, particularly the latter.

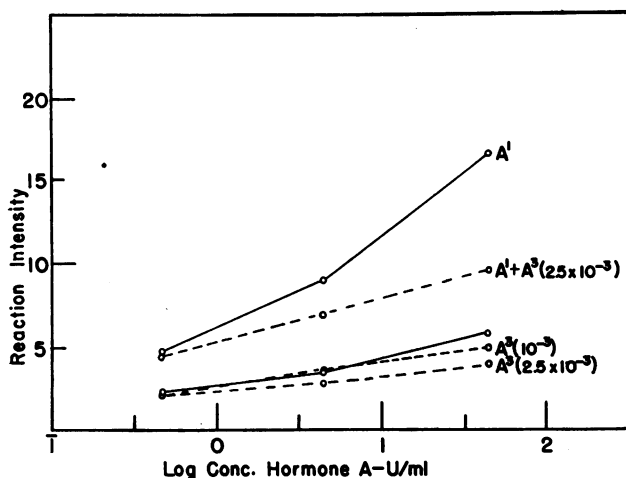


FIGURE 5

Suppression by hormone A^3 of the response induced by graded concentrations of hormone A in the presence and absence of hormone A^1 . Solid curves represent control responses; broken curves, the suppressed responses induced in the presence of hormone A^3 (see text for concentrations).

Hormone A^3 acts to limit the number of antheridial hyphae produced by the plant in response to the other hormones of the *A-Complex*. This inhibition has been found to be effective on the response induced by both hormones A and A^2 and, most markedly, on that induced by hormone A in the presence of an optimal concentration of A^1 . The pattern of inhibition by hormone A^3 of the response induced by hormone A in the presence and absence of added A^1 is shown by the broken curves of figure 5. The indicated concentrations of A^3 are those of an acetone solution of the hormone derived from the filtrate of mass culture of σ^1 mycelium; this solution contained hormone A^3 at 100 times its concentration in the raw

filtrate. The highest concentration used here, one-fourth that of the raw filtrate, significantly reduces the number of antheridial hyphae produced by *A* alone and by *A* plus *A*¹. The slight depression of the reaction by the lower concentration of *A*³ at 50 U./ml. hormone *A* is of doubtful significance.

The effect of hormone *A*³ on the reaction induced by *A*² has been tested only in a preliminary series. On the basis of these tests, a slight but significant decrease in the reaction induced by *A*² results from the addition of *A*³. No attempts have been made to test the effect of *A*³ in more complicated combinations of hormones *A*, *A*¹ and *A*².

The chemical identity of none of the hormones of the *A-Complex* is known; furthermore, but little is known about their chemical and physical properties. Certain of the properties of hormone *A* were described in connection with an unsuccessful attempt to isolate and identify the hormone,³ but insufficient information was obtained even to assign the category of organic compounds to which the hormone belongs. Hormone *A*¹ has been found, in preliminary studies, to be readily dialyzable, neutral in reaction, non-migratory in an electric field, quite stable to heat, acid and oxidizing agents, and destroyed by prolonged alkaline hydrolysis; it is readily soluble in water but no other adequate solvent for it has been found. Nothing whatever is known of hormones *A*² and *A*³ beyond their solubilities in water and acetone (or dioxane), respectively. Chemical characterization and identification of these hormones of the *A-Complex*, as well as the other hormones effective in the sexual process of *Achlya*, have been and will continue to be seriously hampered by the relative difficulty of obtaining the raw materials in sufficient quantities.

The four hormones of the *A-Complex* are secreted by vegetative plants in the presence or absence of sexually compatible strains. Furthermore, sufficient interspecific testing has been done to show that the secretions of ♂ and ♀ strains of at least two heterothallic species, *A. bisexualis* and *A. ambisexualis*, are completely comparable and that the *A*-hormones of either species are completely effective on the ♂ strain of the other species. It does not necessarily follow from this fact that the hormones of the *A-Complex* secreted by the two species are chemically identical.

The titers of three of the four hormones of the *A-Complex* are known to vary characteristically with the age of the cultures in which they are produced. The titer of hormone *A*, in ♀ cultures, remains low for the first week of vegetative growth after which it increases rapidly to reach a maximum at 10–12 days. Hormone *A*¹, in ♂ culture, reaches a maximal concentration three to five days after the initiation of vegetative growth and thereafter steadily decreases to approximately one-half its maximal activity at 14 days. The titer of hormone *A*³, also in ♂ cultures, builds up to a maximum within the first five days of growth and remains constant

thereafter. No specific study has been made of activity of hormone A^2 (φ) in respect to culture age.

A revised scheme for the mechanism of hormonal control in heterothallic *Achlya*, including the two new members of the *A-Complex*, hormones A^2 and A^3 , described here for the first time, is given in figure 6. Only the first of the several stages in the sexual reaction, that pertaining to the production of antheridial hyphae, has been subjected to intense quantitative study and it is not unlikely that the hormonal mechanism, as presented here, will require many further revisions as subsequent stages are successively brought under intensive examination.

Summary.—Two new hormones, A^2 and A^3 , secreted by vegetative mycelia of φ and σ^7 strains, respectively, have been shown to be involved in the initiation of the sexual reaction in heterothallic species of *Achlya*. Thus the quantitative control of the production of antheridial hyphae on σ^7 plants depends upon four distinct hormones: hormones A and A^2 , secreted by the φ , and hormones A^1 (originally designated A') and A^3 , secreted by the σ^7 . These hormones regulate the production of antheridial hyphae in such a way that the intensity of the reaction is: (1) a logarithmic function of the concentration of A , (2) an exponential function⁵ of the concentration of A^2 , (3) a linear function of the concentration of A^1 in the presence of A , (4) increased by A^1 in the presence of A^2 (the precise pattern of augmentation not yet determined), (5) roughly a logarithmic function of the concentration of A^2 in the presence of A or in the combined presence of A plus A^1 , and (6) decreased by A^3 in the presence of A or A^2 alone and in the combined presence of A plus A^1 . A revised diagram of the entire hormonal mechanism, including the above additions to the *A-Complex*, is given.

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¹ Raper, John R., *Science*, **89**, 321 (1939); *Am. J. Bot.*, **26**, 639 (1939); *Ibid.*, **27**, 162 (1940).

² Raper, John R., *Am. J. Bot.*, **29**, 159 (1942); *Proc. Natl. Acad. Sci.*, **28**, 509 (1942).

³ Raper, John R., and Haagan-Smit, A. J., *J. Biol. Chem.*, **143**, 311 (1942).

⁴ Collection data, etc., of these materials are given in: Raper, John R., *Bot. Gaz.* (in press). Cultures of σ^7 and φ strains of *Achlya bisexualis* and *A. ambisexualis*, as well as a number of sexual intergrade strains of the latter species, have been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Holland.

⁵ A plot of log log antheridial hyphae vs. log concentration of A^2 reveals a linear relationship. From this it follows that $I = e^{ax}$, where I is reaction intensity, x , the concentration of A^2 and k , a proportionality constant. The author wishes to thank Dr. Leonard J. Savage for pointing out this relationship.

⁶ An alternate interpretation of A^2 activity might be that the water-soluble fraction of ♀ filtrate contains A^1 plus an effective contamination of A . The pattern of A^1 augmentation on A^2 response and the expected response curve of an $A + A^1$ mixture, when the two hormones are simultaneously diluted, lend strong support for such an hypothesis. On quantitative grounds, however, it is apparent that no possible mixture of hormones A and A^1 could give either the augmentative or inhibitory effects attributed to hormone A^2 when the water-soluble fraction is added to known concentrations of hormone A in the absence and presence of A^1 , respectively (Figs. 3 and 4). It is because of these latter effects that the interpretation presented in this paper is favored over that of a mixture of previously known hormones.

ELECTRON TRANSFER IN INTERMETALLIC COMPOUNDS

BY LINUS PAULING

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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In our discussions of the electronic structure of intermetallic compounds during the last three years brief mention has been made from time to time of the phenomenon of electron transfer.¹⁻³ The interpretation of the observed interatomic distances in many metallic phases seems to require the assumption that electron transfer has taken place; an example is Al_9Co_2 , for which the distances⁴ support the charge distribution⁵ $Al_9^{-2/9}Co_2^{+1}$. The indication by physical properties of a filled-Brillouin-zone structure for Fe_5Zn_{21} also led to the suggestion of electron transfer:² about $1/4$ electron is indicated to have been removed from each zinc atom and about one electron added to each iron atom.

In this paper it is pointed out that the analysis of interatomic distances shows that electron transfer takes place in a great many interatomic compounds, and that the numbers of electrons involved are reasonable, in relation to the changes in valence resulting from loss or gain of electrons and to the partial ionic character of the bonds between unlike atoms and the striving of atoms toward electroneutrality.

Let us divide atoms into three classes: hypoelectronic (electron-deficient) atoms, hyperelectronic (electron-excess) atoms, and buffer atoms. Hypoelectronic atoms are atoms that can increase their valence by adding electrons. The hypoelectronic elements include the first three elements of each short period and the first five elements of each long period, as shown in